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Fig. 1

## DRAP Complete cDNA Clone Insert

```

agcgattacg gtagagatat ggtaatgcaa cggtggatgt gaactccttg      50
gtttgcggtg aatgcgttca acggtggatc ttgccttgca cctttcgcg      100
tagacgacat acggatacag atacagatac agaatggcct ccaacaacag      150
tagtaccacc gatctggaca gccaggtcaa tgtggaggat ttgcccataa      200
cgttcaaggt gaagtacatt ggttccgaag tggcacgtgg cttatggggc      250
attaagtata cgcgtcgtcc ggttgacata atggtggggc tggccaagaa      300
cctgccgccc aataaggtgc tgcccaactg cgaactgaag gtgtccaccg      350
acggagtgcca gctggagatc atatcgccaa aggccagcat caatcactgg      400
agctatccca tcgacacgat ctcgatatggc gttcaggacc tgggtctacac      450
aaggggtcttt gccatgatcg tgggtgaagga cgagtcgagt ccgcatccct      500
ttgaggttca cgccttcgtg tgcgacagtc gtgcgatggc gcggaagttg      550
acctttgccc tggccggccg ctttccagga ttactcgcg      600
aggcaaccgg tgaggaggag ggcgaggcca cgcccagcga cactattaca      650
cccacgcgac acaagttcgc catcgatctg cgaacgccgg agaaatccag      700
gctggcgaac tggagcagga aacggaggcg tagttatcct ggtgatcctg      750
cgttggtcc gtcaatgaga tgtgatgtgt tagttactta acgtccagt      800
ttcactgtat ctgtaaattg tggttctctc acctggtagt tgccctcaca      850
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aaataaatta cgaatatggt atgtttggct atttgaattg ggctacaacc      950
tggtgatatg ccacttgga aaaaaaaaaa acgccagcac caattctttt      1000
acttctgttt cttgtgaccg acataaaaga tgcaccaaag ctgctattcc      1050
accagcgttc tttattccac gcttgttttc atcattttgt cttccgtaag      1100
ataaattacg taaagcacca caggcatttt tatgtatttc tggagaatca      1150
taagatagca gtcgaactaa tggtggtata cctcccagag atcttgtagc      1200
ttgcttggtt ggatcatcca tgtagcaca atgctgtaga taggctgctg      1250
cattagcttt tatagcacta ctcggttgcc ttaaaaagct tattacttct      1300
gaaagatttg gatcccga tctcattgta gaacaaatat cattttctga      1350
tccttcaatg taatcatcct tttcttcc      1378

```

Longest ORF nt 104 - 610

Probable Start Codon nt 134

CDS Expressed as Recombinant Protein nt 134 - 610

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Figure 2

### DRAP Coding Sequence - Restriction Map

With 206 enzymes: \* MaxCuts: 1

B a C j M D  
 I e I e I d  
 I I I I I

134 atggcctccaacaacagtagtaccaccgatctggacagccaggtcaatgtggaggatttg 193  
 -----+-----+-----+-----+-----+-----+-----  
 taccggagggtgtgtgtcatcatggtggctagacctgtcgggtccagttacacctcctaaac

b M A S N N S S T T D L D S Q V N V E D L -

P  
 s  
 p  
 l  
 4 DN rl B sP M HM  
 0 da am w gs  
 6 II Al o ae  
 I IV II I II  
 /

194 cccataacgttcaagggtgaagtacattggttccgaagtgccacgtggccttatggggcatt 253  
 -----+-----+-----+-----+-----+-----+-----  
 ggggtattgcaaggttccacttcatgtaaccaaggcttcaccgtgcaccgaataaccccgtaa

b P I T F K V K Y I G S E V A R G L W G I -

B  
 s  
 t A  
 l f  
 l lM  
 0 Il  
 7 Iu  
 I II  
 /

254 aagtatacgctcgctccggttgacataatggtggcgctggccaagaacctgccgcccaat 313  
 -----+-----+-----+-----+-----+-----+-----  
 ttcatatgcgcgagcgcccaactgtattaccacccgcaccggttcttggaaggcggggtta

b K Y T R R P V D I M V G V A K N L P P N -

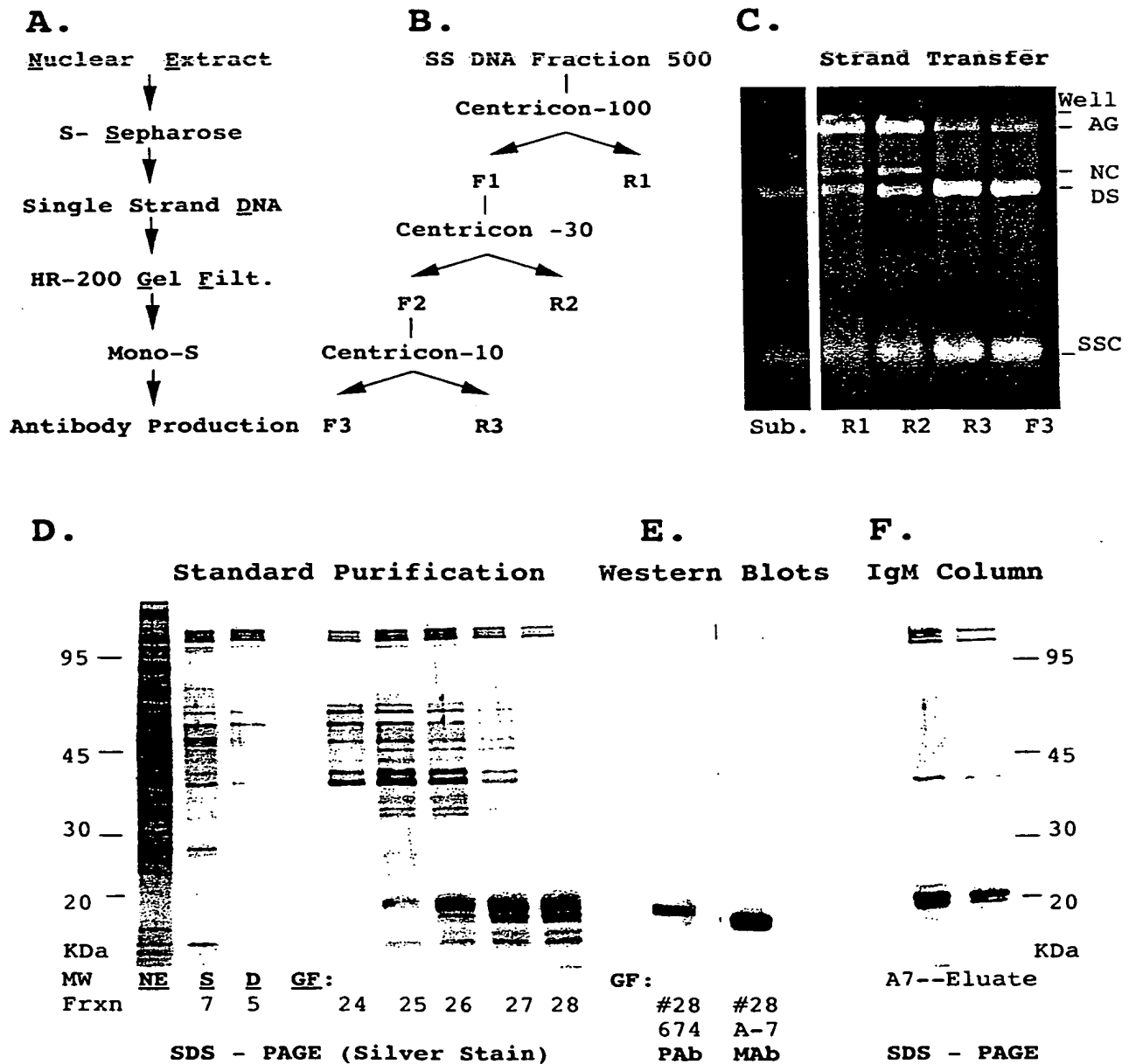
E M  
 c sP  
 T o pv  
 s 5 Au  
 e 7 lI  
 I I II  
 /

314 aagtggtctgcccaactgcgaactgaagggtgtccaccgacggagtccagctggagatcata 373  
 -----+-----+-----+-----+-----+-----+-----  
 ttccacgacgggttgacgcttgacttccacagggtggctgcctcagggtcgacctctagtat

b K V L P N C E L K V S T D G V Q L E I I -



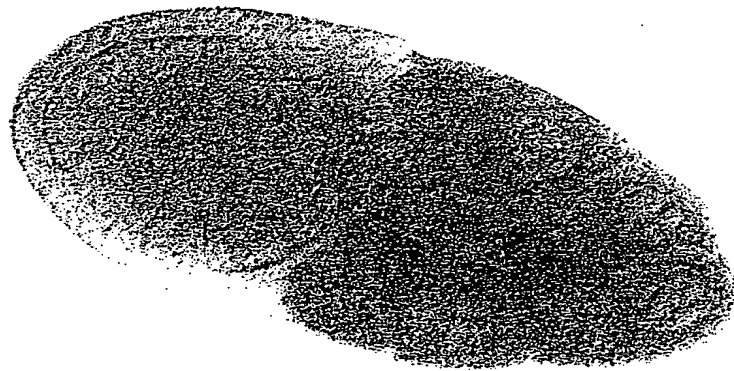
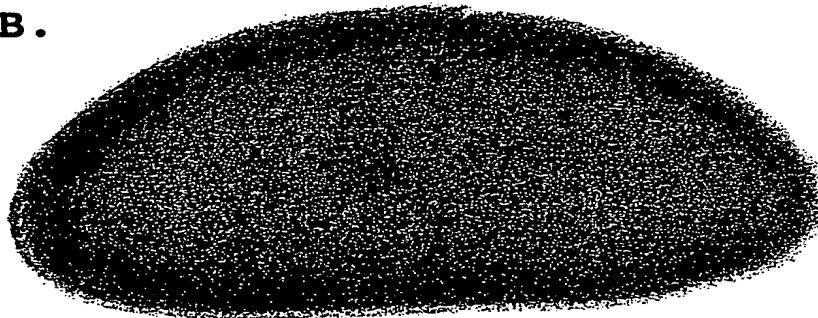
Figures 3A-3F

**Recombination-Associated Protein Purification**

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**DRAP Transcript Distribution****A.****B.**

Figures 5A and 5B

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## Figure 4 A-4D

## Recombination-Associated Protein cDNA Clone

A. DRAP: 1378 bp Eco RI insert  
ORF 104-610

1 134 281 902 970 1378  
ATG ATG AATAAA (A)12 (A)29  
#1 #50



B. MASNNSSTD LDSQVNVEDL PITFKVKYIG SEVARGLWGI KYTRRPVDIM 50  
VGVAKNLPPN KVLPCNELKV STDGVQLEII SPKASINHWS YIPDTISYGV 100  
QDLVYTRVFA MIVVKDESSP HPFEVHAFVC DSRAMARKLT FALAGRLPGL 150  
LATGGGGNR 159

Potential	D1-----(30 to 70)---D2---(35+)---E1	Motifs
AA#s	(10 or 12)---(36 to 38)---48---(30)--- 78	
	( 19 )---( 54 )---73---(44)---117	
	(19 or 48)---(46 to 75)---94---(30)---124	

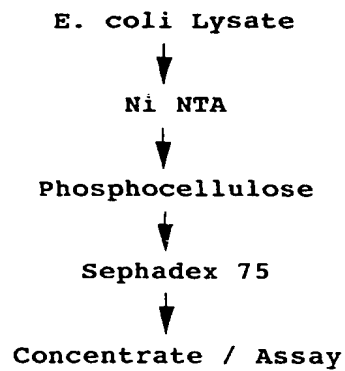
D.

Rad 51	Mouse - Human	L L I V - D - S
Rad 51	Yeast	L I V V - D - S
DCM 1	Yeast	L I V V - D - S
RecA	E. Coli	V I V V - D - S
Drosophila DRAP		M I V V K D E S S P
FLP Recombinase		M I A L K D E T N P
T4 Gene 32 Protein		I L V V K D P A A P
		M I A V - D V E M G E
		K - G F S S E
Human Topoisomerase I		I K D E - - P
		K D G S S E
		G F S S P

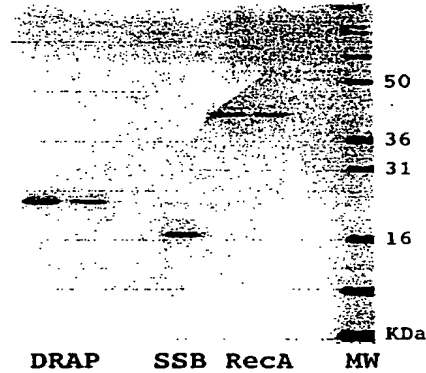
Figures 6A-6F

## Recombination-Associated Protein Activities

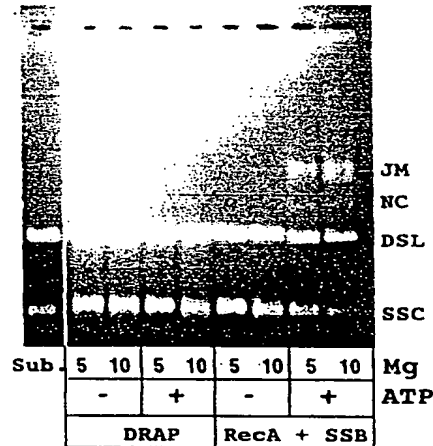
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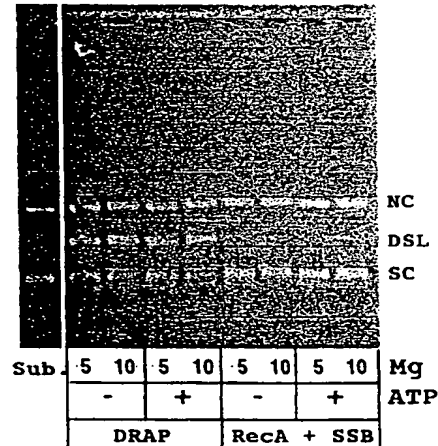
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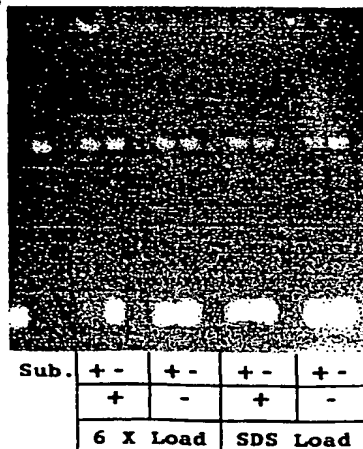
C.



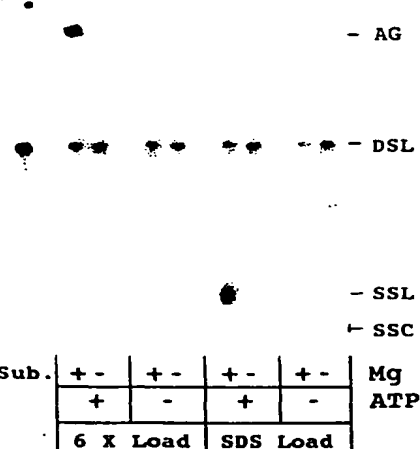
D.



E.



F.

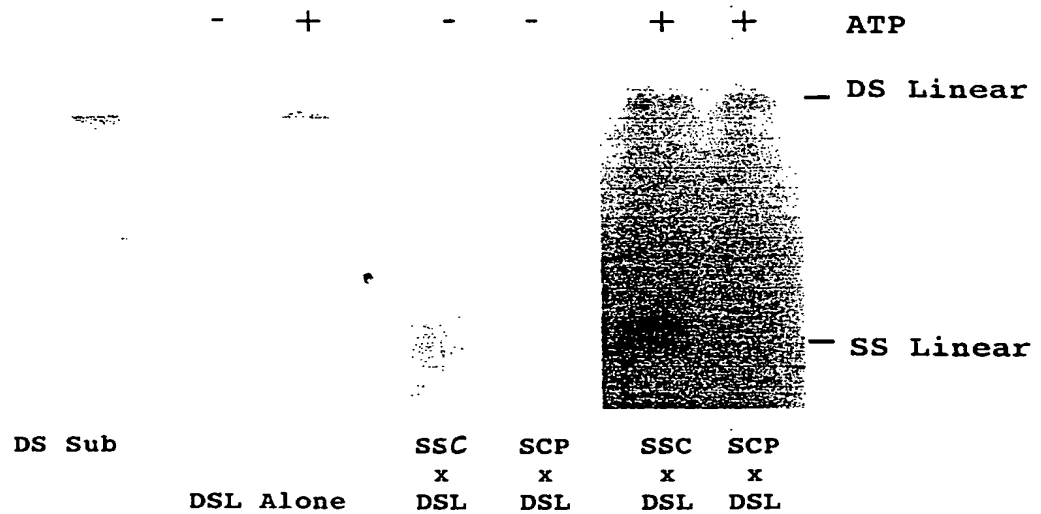


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Figure 7

**ATP-Dependent Three Strand Exchange Reaction**



## Figure 8

Nuclease Assay - 5'  $^{32}\text{P}$  Oligo

(6% 1X TAE PAG)

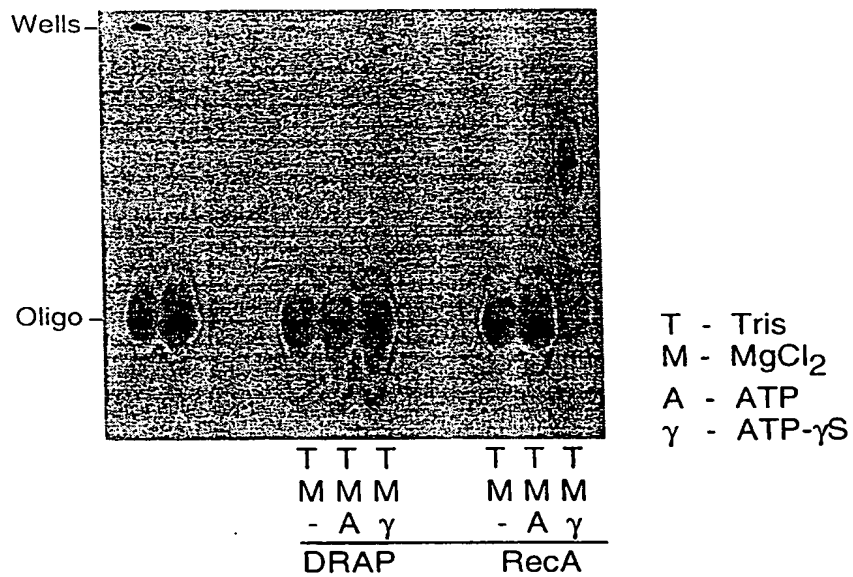
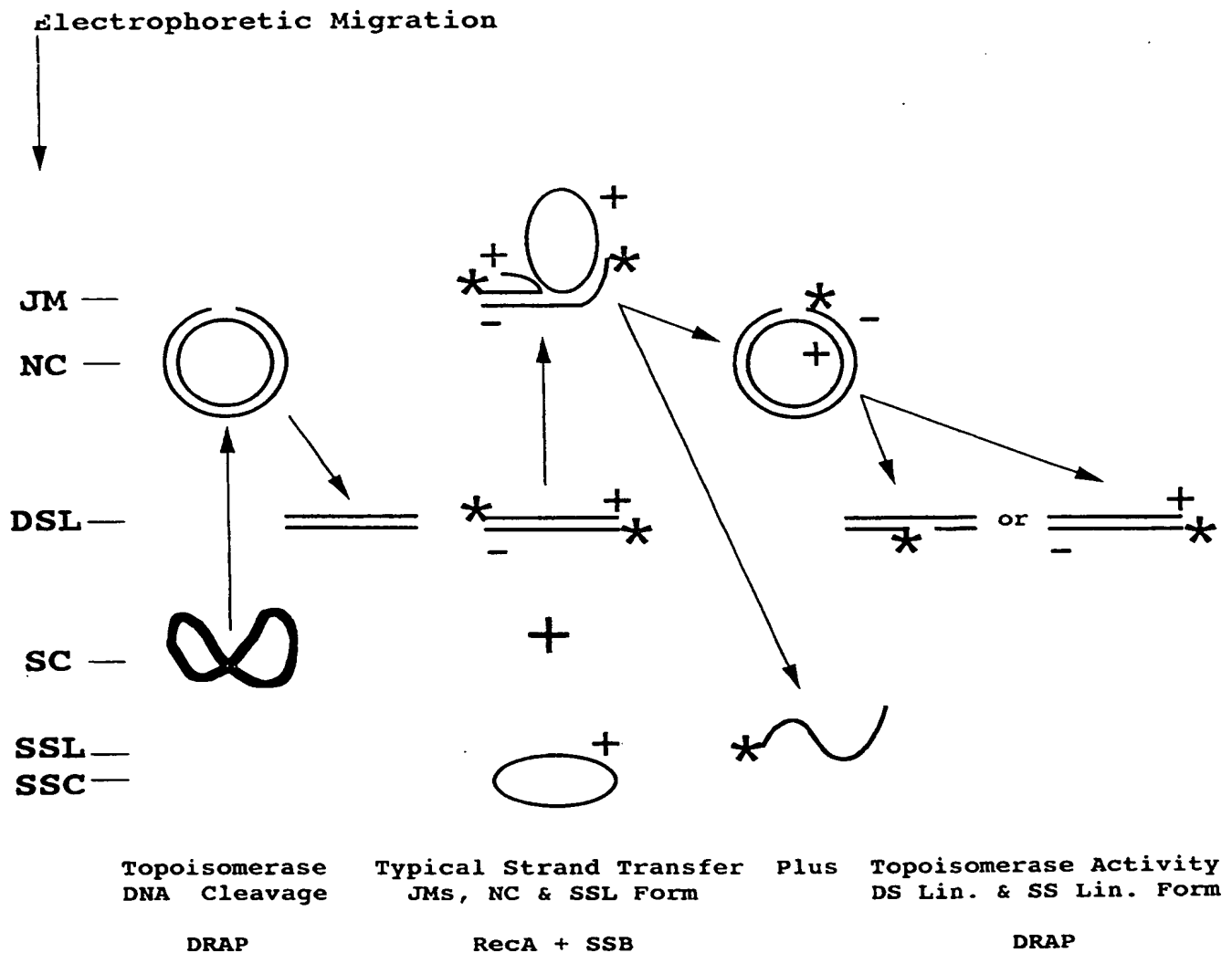
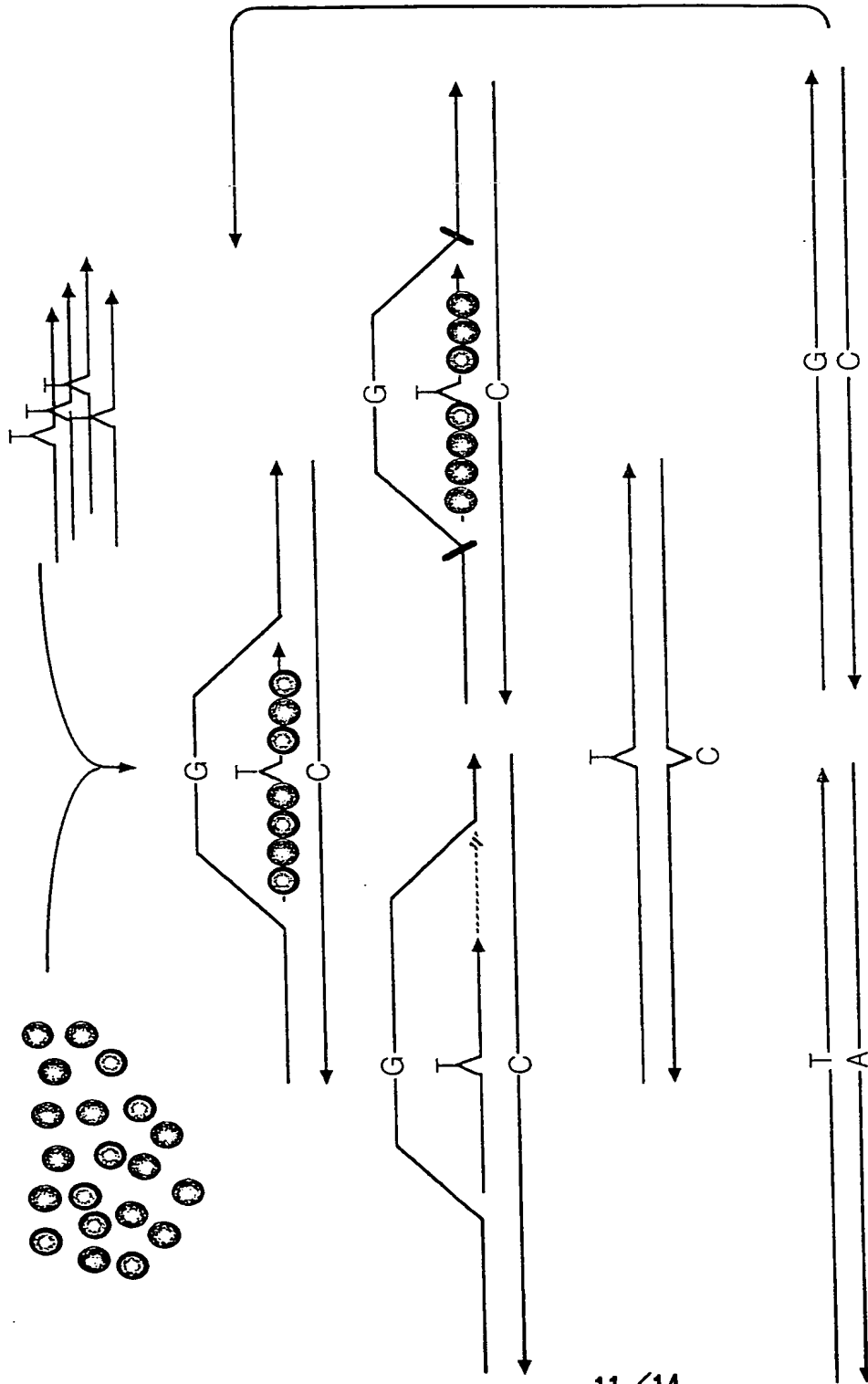


Figure 9

# Strand Transfer and DNA Cleavage Reaction



# Driving A Gene Conversion With A Recombinase and Oligos



# Targeted Transgenics

Gene	Oligo (No Phenotype)	Oligo + DRAP (Molar Ratio - Protein : Oligo)
1. N - myc (Exon 1)	16	Low (1:1) 60 OK High (100:1) 4 Alive 11 Stillborn
2. $\beta$ 1 globin (Ala --> Val)	7	High (100:1) 10 OK 5 Runts 4 OK 1 Sickly
3. Agouti	11	High (100:1) 8 OK

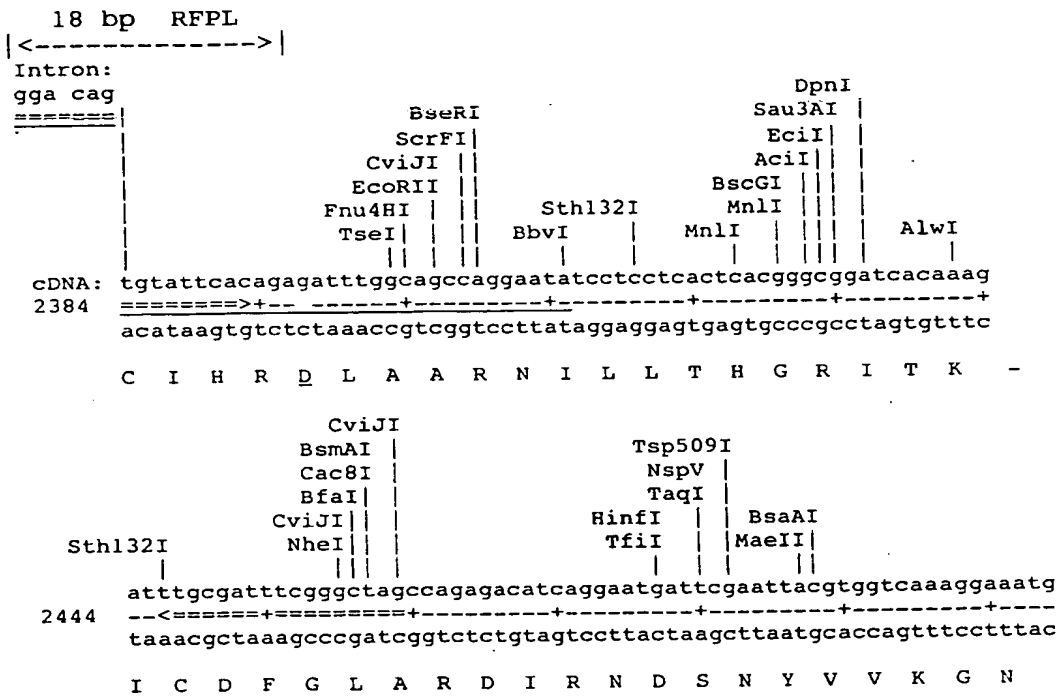
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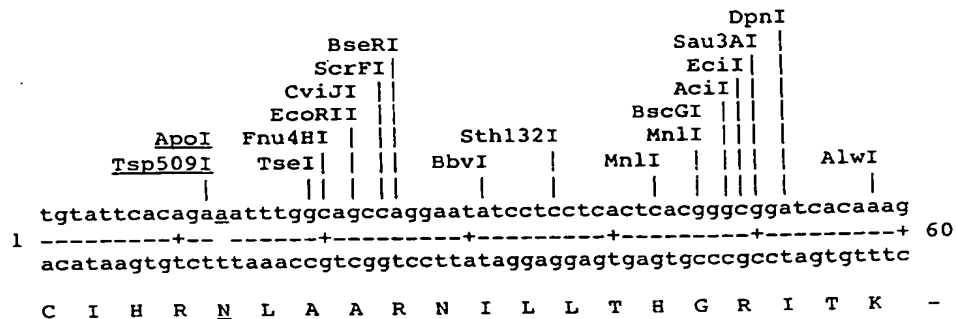
PCT/US00/19901

## Figure 12

### Murine c-Kit Exon 17



### W42 Mutation



Mutation & RFLP

Underline:

=====&gt; &lt;=====

Potential Mutagenic Oligo

Potential PCR Primers for RFLP Analysis

Figure 13

## Targeted Transgenic Mice



White-Spotted Mutant



Control Mouse